

Serial No.: 09/847,960

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IN THE SPECIFICATION:

Please replace the paragraph beginning at page 3, line 14, with the following rewritten paragraph:

A1
In an additional aspect, the present invention provides methods of quantifying the amount of a plurality of germline constructs comprising preparing mRNA from the plurality of cells to form an mRNA mixture, and adding at least three RNase protection probes (RPPs) selected from the group consisting of the sequences depicted in Figures 3A-3B or 4A-4B (SEQ ID NOS:1-13). An RNase protection enzyme (RPE) is added to the mixture, such that mRNA that is not protected is digested, and the amount of each germline mRNA is quantified.

Please replace the paragraph beginning at page 3, line 23, with the following rewritten paragraph:

A2
Figures 3A-3B (SEQ ID NOS:1-6) depict the sequences of some "long" RPPs of the invention, these probes being directed to human immunoglobulin germline transcripts.

Please replace the paragraph beginning at page 3, line 24, with the following rewritten paragraph:

A3
Figures 4A-4B (SEQ ID NOS:7-13) depict the sequences of some "short" RPPs of the invention, these probes being directed to human immunoglobulin germline transcripts.

Please replace the paragraph beginning at page 4, line 1, with the following rewritten paragraph:

A4
Figure 8 depicts a commercially available vector for the production of the RPPs of the invention. The vector circle map of the pSP72 Vector (Promega, Madison, WI) shows the following sequence reference points:

- a. SP6 RNA polymerase transcription initiation site at position 1.
- b. T7 RNA polymerase transcription site at position 101.
- c. SP6 RNA polymerase promoter from position 2446 to position 6.
- d. T7 RNA polymerase promoter from position 2446 to position 6.
- e. multiple cloning sites from position 4 to position 90.

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f. β -lactamase (Amp') coding region from position 1135 to position 1995.

NY
The vector is capable of use for transcription *in vitro* from dual opposed promoters, using the protocol from the Riboprobe® *in vitro* Transcription Systems Technical Manual (# TM061; Promega, Madison, WI). The pSP72 and pSP73 vectors are identical except for the orientation of the multiple cloning region.

Please replace the paragraph beginning at page 18, line 20, with the following rewritten paragraph:

AS
RPA probes include for example the germline Ig α -2 probe depicted in Figure 3A (SEQ ID NO:1). This RPA probe comprises a nucleic acid sequence about 532 nucleotides in length. In a preferred embodiment, the present invention provides Ig α -2 RPA probes consisting essentially of nucleotides from about 1 to about 530 of the Ig α 2 probe depicted in Figure 3A. In another preferred embodiment, the present invention provides Ig α 2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about 470 or about 460 or about 450 or about 440 or about 430 of the Ig α 2 probe depicted in Figure 3A.

Please replace the paragraph beginning at page 18, line 28, with the following rewritten paragraph:

AG
Also provided by the present invention is the germline Ig α -2 probe depicted in Figure 4A (SEQ ID NO:8). This RPA probe comprises a nucleic acid sequence about 430 nucleotides in length. The Ig α -2 probe sequence depicted in Figure 4A is preferred over the Ig α -2 probe sequence depicted in Figure 3A (SEQ ID NO:1). In a preferred embodiment, the present invention provides Ig α -2 RPA probes consisting essentially of nucleotides from about 1 to about 430 of the Ig α 2 probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Ig α 2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 430 or about 425 or about 420 or about 415 of the Ig α 2 probe depicted in Figure 4A.

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Please replace the paragraph beginning at page 19, line 2, with the following rewritten paragraph:

A7
Also provided herein are Ig α -2 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3A (SEQ ID NO:1), which comprise the Ig α -2 nucleic acid sequence depicted in Figure 3 and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig α -2 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers L04541 (being 5') and AL389978 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig α -2 RPA probes which are in addition to the Ig α 2 probe sequence depicted in Figure 3A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of L04541 and AL389978 and contiguous with the preceding complementary sequence.

Please replace the paragraph beginning at page 19, line 11, with the following rewritten paragraph:

A8
RPA probes include for example the germline Ig-epsilon probe depicted in Figure 3A (SEQ ID NO:2). This RPA probe comprises a nucleic acid sequence about 202 nucleotides in length. In a preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 to about 200 of the Ig-epsilon probe depicted in Figure 3A. In another preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 200 or about 195 or about 190 or about 185 of the Ig-epsilon probe depicted in Figure 3A.

Please replace the paragraph beginning at page 19, line 18, with the following rewritten paragraph:

A9
Also provided by the present invention is the germline Ig-epsilon probe depicted in Figure 4A (SEQ ID NO:9). This RPA probe comprises a nucleic acid sequence about 202 nucleotides in length. In a preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 to about 200 of the Ig-epsilon probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 200 or about 195 or about 190 or about 185 of the Ig-epsilon probe depicted in Figure 4A.

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Please replace the paragraph beginning at page 19, line 25, with the following rewritten paragraph:

AJ

Also provided herein are Ig-epsilon RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3A (SEQ ID NO:2), which comprise the Ig-epsilon nucleic acid sequence depicted in Figure 3 and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig-epsilon probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers X56797 (being 5') and J00222 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig-epsilon RPA probes which are in addition to the Ig-epsilon probe sequence depicted in Figure 3A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of X56797 and J00222 and contiguous with the preceding complementary sequence.

Please replace the paragraph beginning at page 20, line 1, with the following rewritten paragraph:

All

RPA probes include for example the germline Ig gamma-1 probe depicted in Figure 3A (SEQ ID NO:3). This RPA probe comprises a nucleic acid sequence about 593 nucleotides in length. In a preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 to about 590 of the Ig gamma-1 probe depicted in Figure 3A. In another preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 590 or about 580 or about 570 or about 560 or about 550 or about 540 or about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about 470 or about 460 or about 450 or about 440 or about 430 or about 420 or about 410 or about 400 or about 390 or about 380 or about 370 of the Ig gamma-1 probe depicted in Figure 3A.

Please replace the paragraph beginning at page 20, line 11, with the following rewritten paragraph:

AJ2

Also provided by the present invention is the germline Ig gamma-1 probe depicted in Figure 4A (SEQ ID NO:10). This RPA probe comprises a nucleic acid sequence about 370 nucleotides in length. The Ig gamma-1 probe sequence depicted in Figure 4A is preferred over the Ig gamma-1 probe sequence depicted in Figure 3A (SEQ ID NO:3). In a preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 to about 370

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A/2

of the Ig gamma-1 probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 370 or about 365 or about 360 or about 355 of the Ig gamma-1 probe depicted in Figure 4A.

Please replace the paragraph beginning at page 20, line 19, with the following rewritten paragraph:

A/3

Also provided herein are Ig gamma-1 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3A (SEQ ID NO:3), which comprise the Ig gamma-1 nucleic acid sequence depicted in Figure 3A and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-1 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers AL122127 (being 5') and Z17370 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-1 RPA probes which are in addition to the Ig gamma-1 probe sequence depicted in Figure 3A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of AL122127 and Z17370 and contiguous with the preceding complementary sequence.

Please replace the paragraph beginning at page 20, line 28, with the following rewritten paragraph:

A/4

RPA probes include for example the germline Ig gamma-2 probe depicted in Figure 3B (SEQ ID NO:4). This RPA probe comprises a nucleic acid sequence about 632 nucleotides in length. In a preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 to about 630 of the Ig gamma-2 probe depicted in Figure 3B. In another preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 630 or about 620 or about 610 or about 600 or about 590 or about 580 or about 570 or about 560 or about 550 or about 540 or about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about 470 or about 460 or about 450 or about 440 or about 430 or about 420 or about 410 or about 400 or about 390 or about 380 of the Ig gamma-2 probe depicted in Figure 3B.

Please replace the paragraph beginning at page 21, line 3, with the following rewritten paragraph:

A15

Also provided by the present invention is the germline Ig gamma-2 probe depicted in Figure 4B (SEQ ID NO:11). This RPA probe comprises a nucleic acid sequence about 387 nucleotides in length. The Ig gamma-2 probe sequence depicted in Figure 4B is preferred over the Ig gamma-2 probe sequence depicted in Figure 3B (SEQ ID NO:4). In a preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 to about 385 of the Ig gamma-2 probe depicted in Figure 4B. In another preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10 to about 385 or about 380 or about 375 or about 370 of the Ig gamma-2 probe depicted in Figure 4B.

Please replace the paragraph beginning at page 21, line 11, with the following rewritten paragraph:

A16

Also provided herein are Ig gamma-2 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3B (SEQ ID NO:4), which comprise the Ig gamma-2 nucleic acid sequence depicted in Figure 3B and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-2 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers U39934 (being 5') and J00230 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-2 RPA probes which are in addition to the Ig gamma-2 probe sequence depicted in Figure 3B comprise a nucleic acid sequence which is additionally complementary to the fused sequence of U39934 and J00230 and contiguous with the preceding complementary sequence.

Please replace the paragraph beginning at page 21, line 20, with the following rewritten paragraph:

A17

RPA probes include for example the germline Ig gamma-3 probe depicted in Figure 3B (SEQ ID NO:5). This RPA probe comprises a nucleic acid sequence about 650 nucleotides in length. In a preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 to about 650 of the Ig gamma-3 probe depicted in Figure 3B. In another preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 650 or about 640 or about 630 or about 620 or about 610 or about 600 or about 590 or about 580 or about 570 or about 560 or about 550 or about 540 or about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about

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470 or about 460 or about 450 or about 440 or about 430 or about 420 or about 410 or about 400 or about 390 of the Ig gamma-3 probe depicted in Figure 3B.

Please replace the paragraph beginning at page 21, line 30, with the following rewritten paragraph:

A18
Also provided by the present invention is the germline Ig gamma-3 probe depicted in Figure 4B (SEQ ID NO:12). This RPA probe comprises a nucleic acid sequence about 391 nucleotides in length. The Ig gamma-3 probe sequence depicted in Figure 4B is preferred over the Ig gamma-3 probe sequence depicted in Figure 3B (SEQ ID NO:5). In a preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 to about 390 of the Ig gamma-3 probe depicted in Figure 4B. In another preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 390 or about 385 or about 380 or about 375 of the Ig gamma-3 probe depicted in Figure 4B.

Please replace the paragraph beginning at page 22, line 4, with the following rewritten paragraph:

A19
Also provided herein are Ig gamma-3 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3B (SEQ ID NO:5), which comprise the Ig gamma-3 nucleic acid sequence depicted in Figure 3B and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-3 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers AL122127 (being 5') and X16110 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-3 RPA probes which are in addition to the Ig gamma-3 probe sequence depicted in Figure 3B comprise a nucleic acid sequence which is additionally complementary to the fused sequence of AL122127 and X16110 and contiguous with the preceding complementary sequence.

Please replace the paragraph beginning at page 22, line 13, with the following rewritten paragraph:

A20
RPA probes include for example the germline Ig gamma-4 probe depicted in Figure 3B (SEQ ID NO:6). This RPA probe comprises a nucleic acid sequence about 706 nucleotides in length. In a preferred

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A20 embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 to about 705 of the Ig gamma-4 probe depicted in Figure 3B. In another preferred embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 705 or about 695 or about 685 or about 675 or about 665 or about 655 or about 645 or about 635 or about 625 or about 615 or about 605 or about 595 or about 585 or about 575 or about 565 or about 555 or about 545 or about 535 or about 525 or about 515 or about 505 or about 495 of the Ig gamma-4 probe depicted in Figure 3B.

Please replace the paragraph beginning at page 22, line 22, with the following rewritten paragraph:

A21 Also provided by the present invention is the germline Ig gamma-4 probe depicted in Figure 4B (SEQ ID NO:13). This RPA probe comprises a nucleic acid sequence about 497 nucleotides in length. The Ig gamma-4 probe sequence depicted in Figure 4B is preferred over the Ig gamma-4 probe sequence depicted in Figure 3B (SEQ ID NO:6). In a preferred embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 to about 495 of the Ig gamma-4 probe depicted in Figure 4B. In another preferred embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 495 or about 490 or about 485 or about 480 of the Ig gamma-4 probe depicted in Figure 4B.

Please replace the paragraph beginning at page 22, line 30, with the following rewritten paragraph:

A22 Also provided herein are Ig gamma-4 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3B (SEQ ID NO:6), which comprise the Ig gamma-4 nucleic acid sequence depicted in Figure 3B and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-4 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers X56796 (being 5') and K01316 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-4 RPA probes which are in addition to the Ig gamma-4 probe sequence depicted in Figure 3B comprise a nucleic acid sequence which is additionally complementary to the fused sequence of X56796 and K01316 and contiguous with the preceding complementary sequence.

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Please replace the paragraph beginning at page 23, line 5, with the following rewritten paragraph:

AJ3

RPA probes include for example the germline Ig α -1 probe depicted in Figure 4A (SEQ ID NO:7). This RPA probe comprises a nucleic acid sequence about 400 nucleotides in length. In a preferred embodiment, the present invention provides Ig α -1 RPA probes consisting essentially of nucleotides from about 1 to about 400 of the Ig α -1 probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Ig α -1 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 400 or about 395 or about 390 or about 385 of the Ig α -1 probe depicted in Figure 4A.

Please replace the paragraph beginning at page 23, line 12, with the following rewritten paragraph:

AJ4

Also provided herein are Ig α -1 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 4A (SEQ ID NO:7), which comprise the Ig α -1 nucleic acid sequence depicted in Figure 4A and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig α -1 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers L04541 (being 5') and BC005951 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig α -1 RPA probes which are in addition to the Ig α -1 probe sequence depicted in Figure 4A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of L04541 and BC005951 and contiguous with the preceding complementary sequence.

Please replace the paragraph beginning at page 23, line 28, with the following rewritten paragraph:

AJ8

Preferred probe sequences of the invention are shown in the figures. Figures 3A-3B (SEQ ID NOS:1-6) depict[s] some "longer" probes and Figures 4A-4B (SEQ ID NOS:7-13) some shorter, preferred versions. Thus, preferred probes include nucleic acids consisting essentially of the sequences shown in Figures 3A-3B or 4A-4B.